Mechanophysiology of cupulae and hair cells in the lateral line of fish and pitch perception of complex sounds in humans

Wiersinga-Post, Jenny Esther Clasine

Chapter 3

Comparative study of frequency selectivity of hair cells in the lateral line of the ruffe and knife fish

J. Esther C. Wiersinga-Post and Sietse M. van Netten

Abstract

In three different fish species, the ruffe (*Acerina cernua* L.), the African knife fish (*Xenomystus nigri*) and the clown knife fish (*Notopterus chitala*), the mechanical filtering properties of cupulae in the supraorbital lateral line canal were measured *in vivo* together with the evoked extracellular AC-receptor potentials. Cut-off frequencies of the mechanical frequency responses of the cupulae were approximately 110 Hz in the ruffe and approximately 400 and 235 Hz in the African knife fish and clown knife fish, respectively. The frequency selectivity functions of the hair cells, derived by dividing the response of the extracellular receptor potential by the mechanical response, were found to have (low-pass) cut-off frequencies of approximately 200 Hz in the ruffe and approximately 320 and 345 Hz in the African knife fish and clown knife fish. The results thus show that the frequency selectivity of cupular mechanics and the frequency selectivity of hair cell filtering are correlated. This correlated frequency selectivity of both subsequent stages of peripheral signal processing suggests that these characteristics are evolved to perceive relevant stimulus frequencies.

1. Introduction

The acoustico-lateralis sensory system of vertebrates is a mechanosensory system which contains organs specialised in the detection of sound (the cochlea in mammals or papillae in lower vertebrates), body acceleration and rotation (the vestibular organ) and fluid motion (the lateral line organ in fishes and aquatic amphibians). The sensory cells in this system are hair cells, which contain a hair bundle protruding from the apical side of the cell. In all organs, the deflection of these hair cell bundles is the actual stimulus for the sensory cells. Peripheral receptive structures, mechanically attached to the hair bundles, transform the various mechanical stimuli, usually via a hydrodynamic interface, into the deflection of the hair cell bundles.

The hair cells of the lateral line organ are grouped in neuromasts, which are distributed over the head and body and are located in the skin surface (superficial neuromasts) or in sub-epidermal bony canals (canal neuromasts). The hair bundles of the hair cells protrude into the peripheral receptive structure, the cupula (Dijkgraaf, 1963; Flock, 1967). Therefore, cupular motion, evoked by fluid motion, results in the deflection of the hair cell bundles.

Measurements of the extracellular receptor potentials of the lateral line hair cells or of the neural activity of their afferents show that superficial neuromasts are sensitive to fluid velocities up to frequencies in the range of 10 to 70 Hz (Görner, 1963; Kroese *et al.*, 1980; Münz, 1985; Kroese and Schellart, 1992) and that canal neuromasts are sensitive to fluid

accelerations (Kalmijn, 1989; Kroese and Schellart, 1992) up to frequencies in the range of 50 to 200 Hz (Kuiper, 1956; Harris and van Bergeijk, 1962; Russell and Lowe, 1983; Münz, 1985; Montgomery and MacDonald, 1987; Wubbels, 1988; Kroese and van Netten, 1989; Coombs and Montgomery, 1992). These filtering characteristics may be attributed to either the peripheral receptive structures (canal, cupula), or to the tuning properties of the hair cells, or both.

In the lateral line of the ruffe, the mechanical filtering properties of the cupula have been studied extensively. In terms of iso-acceleration stimuli (e.g. Kalmijn, 1989), the (low-pass) cut-off frequency of the cupular frequency response was found to be approximately 110 Hz (van Netten and Kroese, 1987). Experiments, in which the mechanical frequency response of the cupula together with the extracellular receptor potentials of the hair cells were measured, showed that the filtering properties of the hair cells start to attenuate the AC-receptor potential about one octave above this mechanical cut-off frequency (Kroese and van Netten, 1987). In the lateral line of the African knife fish, mechanical cut-off frequencies of about 375 Hz were measured (van Netten *et al.*, 1994). This mechanical cut-off frequency exceeds the highest cut-off frequencies of 150 to 200 Hz for hair cell activity or afferent nerve fibre activity in the lateral line described thus far (e.g. Harris and van Bergeijk, 1962; Münz, 1985). Therefore, it is interesting to know whether the hair cells in the lateral line of the knife fish can process the relatively high frequencies with which they can be stimulated via the cupula.

In the present study, we describe *in vivo* measurements of the mechanical frequency responses of the cupulae and hair cell activity of neuromasts in the lateral line of the ruffe, the African knife fish and clown knife fish. The results show that not only for the ruffe, but also for the knife fishes the frequency selectivity of cupular mechanics and hair cell activity are correlated.

2. Methods

In three different fish species, the ruffe (*Acerina cernua* L.), the African knife fish *(Xenomystus nigri*) and the clown knife fish (*Notopterus chitala*), the mechanical frequency response of the cupula together with the evoked response of the mechanically coupled hair cells were measured in the neuromast located above the orbit in the supraorbital canal (neuromast no. 3 in the ruffe, numbering following Jakubowski, 1963; for a description of the lateral line in the knife fish, see Kapoor, 1964; Sharma, 1964).

(a) Preparation

Six ruffe (body length: 11 to 13 cm), four African knife fishes (body length: 13 to 15 cm) and two clown knife fishes (body length: 10 to 15 cm) were used for this study. Fish were anaesthetised with an intraperitoneal injection of Saffan (Pitman Moore, ca 50 mg/kg body weight) and placed in a fish tank where they were artificially respired with a flow of tap water through the gills and held rigidly in place with head and body clamps. The temperature of the water was 15 ^{o}C (ruffe) and 25 ^{o}C (knife fishes).

The supraorbital canal neuromast located above the orbit was exposed by removing the skin overlying the neuromast. The morphology of the supraorbital canal of the ruffe and the knife fishes differ in that bony bridges cover the cupulae in the ruffe, while they are absent

in both types of knife fishes. In the ruffe, the bony bridge covering neuromast no. 3 was removed in order to get access to the neuromast. In the African knife fish, only a small piece of bone, protruding from the canal wall, was removed.

An incident light polarizing microscope (ILPM, Kroese and van Netten, 1987) was used to visualise the maculae and to focus the laser beams of a laser interferometer on spots in the cupula (see section c). Blood flow through the macula was well visible and was used to monitor the condition of a neuromast.

(b) Mechanical stimulation

Neuromasts were stimulated with canal fluid motion produced by a small sphere (Ø 0.8 mm) placed inside the canal about 4 mm rostral of the cupula. The sphere was moved sinusoidally in the direction of the longitudinal axis of the canal at several frequencies in the range of 20 to 360 Hz (ruffe), or 100 to 1000 Hz (knife fishes). The amplitude of the displacement of the stimulus sphere was usually about 3 µm in the experiments with the ruffe and about 1 µm with the knife fishes. Stimuli evoked extracellular receptor potentials well below saturation.

(c) Measurement of cupular motion

Cupular motion was measured as a function of stimulus frequency with a differential laser interferometer coupled to the ILPM. The optical configuration of the laser interferometer was as described by van Netten (1988; see also chapter 1).

For the detection of cupular motion, backscattered laser light from an optical irregularity in the cupula was used. Backscattered light was measured by a photomultiplier. Its signal was demodulated with a modified frequency demodulator (Polytec, OFV 3000). The

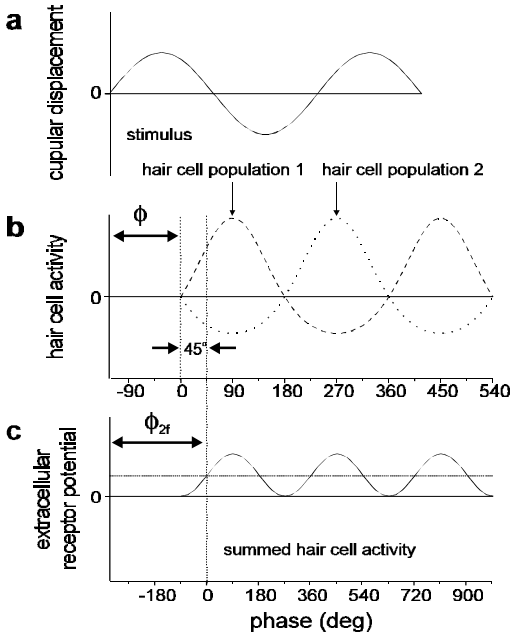

Figure 1. Schematic representation of the formation of the extracellular receptor potential. **(a)** Stimulus input. **(b)** Non linear response of the two opositely oriented hair cell populations. **(c)** The extracellular receptor potential, shaped by the summed activity of all hair cells. Its main component is produced at twice the stimulus frequency and lags the phase of one hair cell population with 45 degrees.

output signal of the demodulator is a linear and calibrated measure of the velocity of the cupula within the range of 10^{-1}-10^{3} µm/s. The velocity signal was low-pass filtered with an 8 pole Bessel filter (Frequency Devices) and amplified before it was digitised with a 16 bit A/D converter (Ariel, DSP-16) at a sampling frequency of 64x (ruffe) or 16x (knife fish) the stimulus frequency. To prevent aliasing, the cut-off frequency of the low-pass filter was set at 8x (ruffe) or 4x (knife fish) the stimulus frequency. Responses to stimuli consisting of 16 periods were 20x averaged before they were saved to disk. The first 3 seconds of every stimulus were not recorded to avoid contamination with transients. The amplitude and phase of the response component of cupular displacement at the stimulus frequency were calculated based on an FFT of the averaged velocity response of cupular motion.

(d) Measurement of extracellular receptor potentials

Extracellular receptor potentials were measured using a silver wire electrode (Ø 0.3 mm), insulated except for the tip, which was placed in the canal at a distance of approximately 0.6 mm from the cupula at its caudal side. An AgCl coated reference electrode was placed in the trunk of the fish. The signal was fed into an AC coupled, differential preamplifier (PARC 113) and further amplified to give a total gain of 10^{4}. The signal was low-pass filtered with a 16 pole Elliptic filter (Difa) with a cut-off frequency at 16x (ruffe) or 8x (knife fish) the stimulus frequency. Signals were averaged and recorded similarly to the mechanical responses. The main component of the extracellular receptor potentials is produced at twice the stimulus frequency, due to combined activation of two oppositely oriented populations of hair cells (Kuiper, 1956; Flock and Wersäll, 1962; Flock, 1971). The amplitude and phase of this component was extracted using an FFT. The phase lags the phase of one hair cell population with 45 degrees and shifts twice as fast (see Fig. 1). Its phase was therefore corrected to obtain the phase representative for one population of hair cells using the formula (see also Fig. 1):

Figure 2. (right) Amplitude and phase of cupular displacements (open symbols, left y-axes) and extracellular receptor potentials (closed symbols, right y-axes) as a function of iso-acceleration stimulus frequencies for two different fish species: the ruffe (squares) and the African knife fish (circles). The phase of the extracellular receptor potential represents the phase of one population of oppositely oriented hair cells. Solid lines represent fits to the measured frequency responses derived from a hydrodynamic model for cupular mechanics (van Netten, 1991). This model describes the displacement of the cupula (X_0) as a function of fluid motion in the canal (D_0) as follows:

$$X_0 = \frac{-i(f/f_t) - \frac{1}{2}\sqrt{2}(i-1)(f/f_t)^{3/2} + \frac{1}{3}(f/f_t)^2}{P_c + i(f/f_t) + \frac{1}{2}\sqrt{2}(i-1)(f/f_t)^{3/2} - \frac{1}{3}(f/f_t)^2} \cdot \frac{D_0}{f^2}$$, with $f_t = \frac{m}{2pra^2}$ and $P_c = \frac{Sar}{6pm^2}$,

where ρ is the fluid density, η is the dynamic viscosity, a is the cupular radius, *S* is the sliding stiffness and D_0 is a scale factor for the amplitude of the canal fluid displacement. The factors D_0 and f_t and P_c are the only free parameters in the model and characterise the frequency response completely. These parameters were fit by eye to the measured data (D_0 = 110, 80 nm, f_t = 12, 47 Hz and P_c = 40 for the ruffe and knife fish, respectively). The cut-off frequencies (f_{cm}) of the fits are 98 Hz for the ruffe and 380 Hz for the African knife fish.

***** For clarity, the plotted amplitude of the extracellular receptor potential of the African knife fish was multiplied with a factor 10.

$j = (j_{2f} + 90^{o}) / 2$.

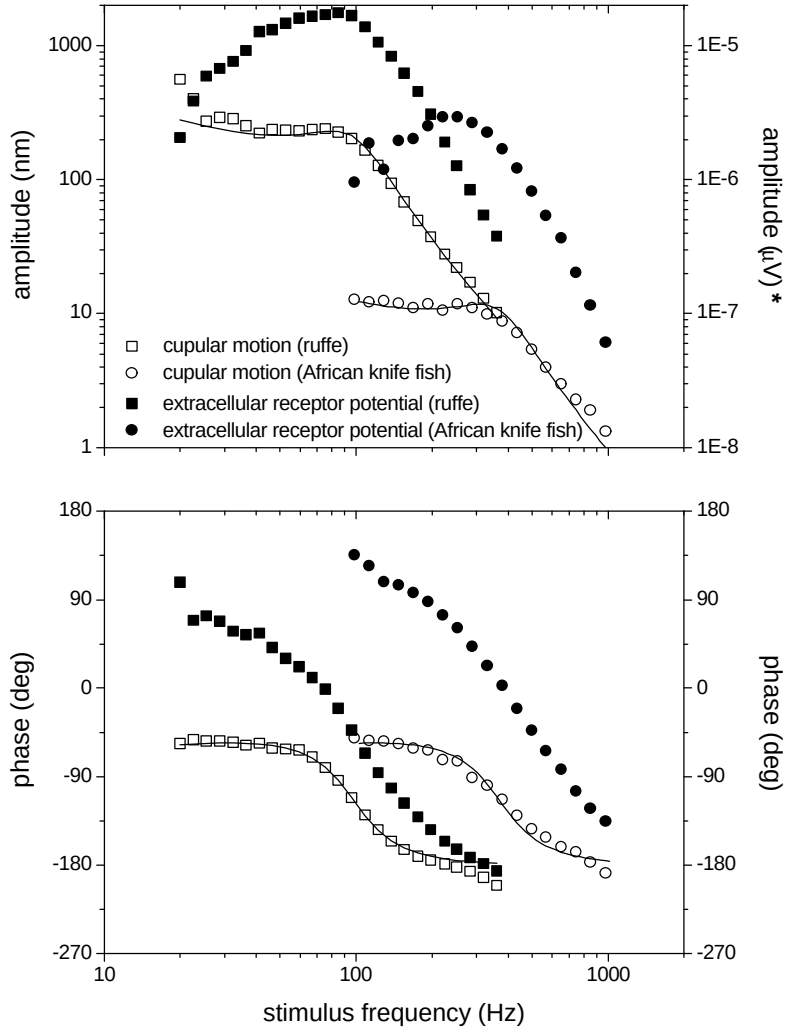
amplitude (nm)
1000
100
10
1
1E-5
1E-6
1E-7
1E-8
amplitude (µV) *
cupular motion (ruffe)
cupular motion (African knife fish)
extracellular receptor potential (ruffe)
extracellular receptor potential (African knife fish)
phase (deg)
180
90
0
-90
-180
-270
phase (deg)
10
100
1000
stimulus frequency (Hz)

Table I. Mean values and standard deviations of the cut-off frequencies of the mechanical frequency responses of the cupulae ($f_{c,m}$), and the (low-pass) cut-off frequencies of the hair cell's frequency selectivity functions ($f_{c,h}$) for the three fish species.

fish species		$f_{c,m}$ (Hz)	$f_{c,h}$ (Hz)
ruffe	(N= 6)	110 ± 11	200 ± 20
African knife fish	(N= 4)	400 ± 74	320 ± 20
clown knife fish	(N= 2)	235 ± 81	345 ± 25

The hair cell's frequency selectivity function was obtained by dividing the component of the extracellular receptor potential by the component of cupular motion.

3. Results

Figure 2 shows typical examples of the amplitude and phase of the mechanical frequency responses (open symbols) and the extracellular receptor potential (closed symbols) measured in the ruffe (squares) and the African knife fish (circles). To show the acceleration detection characteristics and the related low pass filtering of the cupular frequency response (e.g. Kalmijn, 1989), responses are plotted as a function of iso-acceleration stimulus frequencies (referenced to 100 Hz, i.e., measured amplitudes were multiplied by 100^2 / f_{stim}^2, with f_{stim} = stimulus frequency). The solid lines show the fits of a hydrodynamic model for cupular motion (van Netten, 1991, see legend of Fig. 2) to the measured responses. Fig. 2 clearly shows that in the knife fish both the cut-off frequency of the mechanical frequency response ($f_{c,m}$) and the frequency of maximal sensitivity of the extracellular receptor potentials are about a factor 3 to 4 higher than in the ruffe.

For all measured mechanical frequency responses a fit of the hydrodynamic model to the data was made and was used to determine the mechanical cut-off frequency, $f_{c,m}$. Results of the mean values of $f_{c,m}$ for the three fish species are shown in the second column of Table I.

The frequency selectivity functions of the hair cells were determined by dividing the measured extracellular receptor potentials by the mechanical responses of the cupula. Figure 3 shows the amplitude (left) and phase (right) of the frequency selectivity functions for (a) the ruffe (N = 6), (b) the African knife fish (N = 4) and (c) the clown knife fish (N = 2). The functions have band filter characteristics. The phase plots show that the phase lead decreases monotonically from low to high stimulus frequencies. The mean values of the (low-pass) cut-off frequencies of hair cell activity ($f_{c,h}$) are shown in the third column of Table I. Figure 3 and Table I clearly show that these cut-off frequencies are significantly higher in the knife fishes as compared to that in the ruffe. The ratio between the cut-off frequencies of the ruffe and the knife fishes is approximately 1.5 to 1.6.

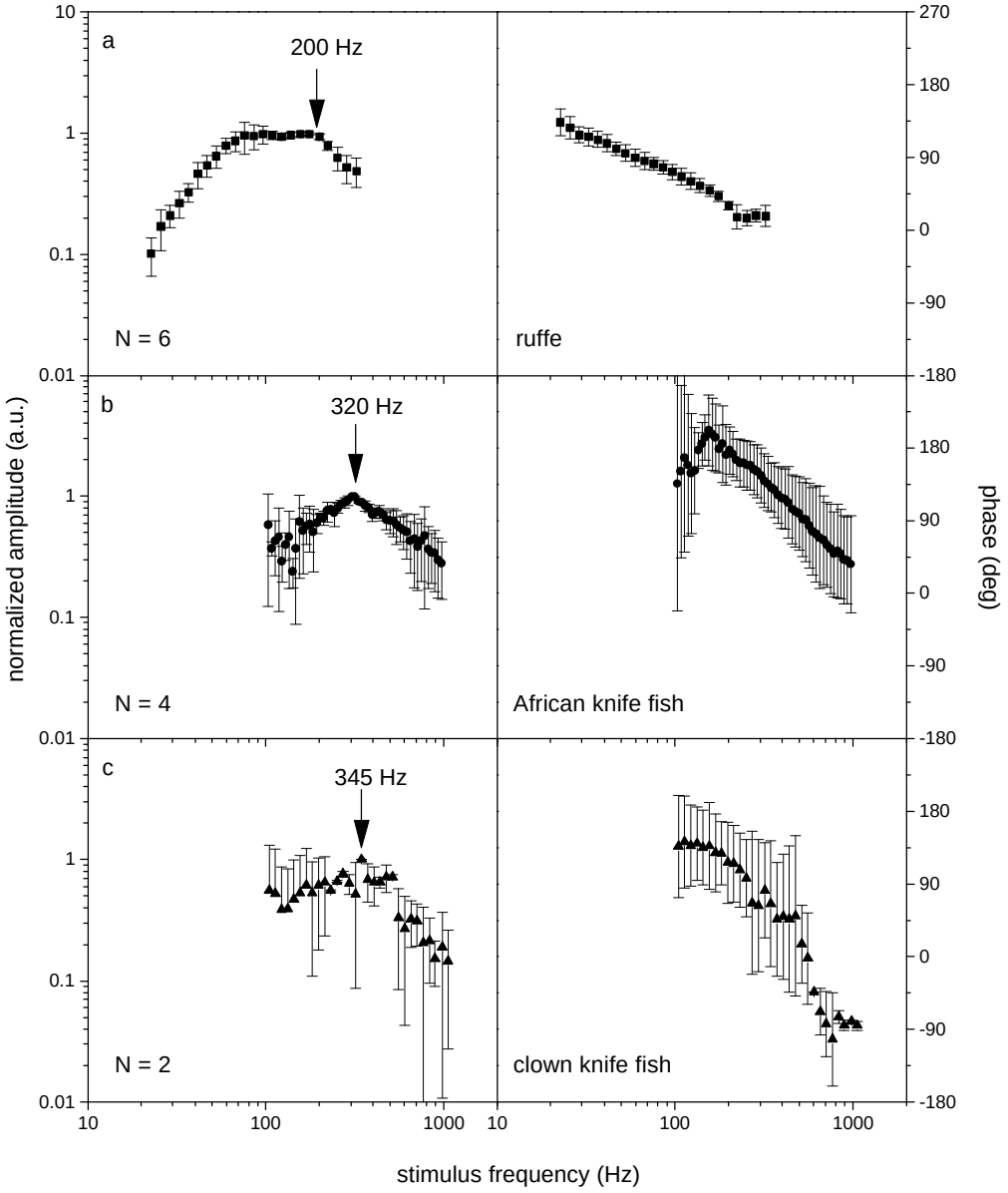

Figure 3. Frequency selectivity functions (amplitude-plot, left; phase-plot right) of hair cells in **(a)** the ruffe, **(b)** the African knife fish and **(c)** the clown knife fish. The functions are obtained by dividing the component of the extracellular receptor potential by the component of the mechanical frequency response. Functions, determined for individual fish, were shifted along the frequency axis as to put the (low-pass) cut-off frequency at the mean cut-off frequency (Table I, third column). Further the functions were normalized. Mean values with standard deviations of respectively 6, 4 and 2 experiments are shown. The (low-pass) cut-off frequencies are indicated by arrows.

4. Discussion

The present experiments show that cupular mechanics as well as hair cell activity in the supraorbital lateral line canal of the knife fish is tuned to relatively high frequencies (between 200 and 400 Hz). This frequency range exceeds the highest best frequency for hair cell activity or lateral line afferent responses described so far (e.g. Münz, 1985).

As shown in Fig. 2, the frequency selectivity functions of the hair cells have band filter characteristics. The slope at low frequencies has been attributed to the adaptation of the hair cells (Corey and Hudspeth, 1983a; Kroese and van Netten, 1989). In terms of first-order filter characteristics, slopes of 20 dB/decade are expected combined with a phase difference between low and high frequencies of 90 degrees. Except for the slope at low frequencies in the ruffe, the amplitude slopes are, indeed, approximately 20 dB/decade. The phase behaviour is, however, not easily interpreted in terms of first order filter characteristics.

In the knife fishes, the extended bandwidths of hair cell frequency selectivity, characterized by the (low-pass) cut-off frequency, $f_{c,h}$, correlates with their cupulae being able to detect a broader range of frequencies of canal fluid acceleration, characterized by the mechanical cut-off frequency, $f_{c,m}$. This suggests that the frequency selective properties of both the cupula and the hair cells are evolved to extract higher stimulus frequencies (up to 300 to 400 Hz) from natural stimuli.

The present results differ from the results of Coombs and Montgomery (1992), who found a large discrepancy between the bandwidths of modelled frequency response properties of cupulae and the measured frequency response properties of afferent nerve fibres. They suggest that the hair cells or the synapse transitions between hair cells and afferent nerve fibres must attenuate the high frequencies with which the cupula stimulates the hair cell bundles, and that the frequency selective properties of the cupulae are not reflected in the afferent responses. In the present experiments no afferent nerve fibre responses were measured. However, the similarity between frequency responses of extracellular receptor potentials and afferent nerve fibre responses, as measured in the ruffe (Wubbels, 1990), indicates that the tuning of the extracellular responses are representative for that of afferent responses. Our results therefore indicate that the mechanical frequency selective properties of the cupulae in the ruffe and the two knife fish species are functional.

In the knife fish the sensitivity of the lateral line neuromast to high frequencies may also facilitate the detection of acoustic stimuli (i.e. underwater sound), since the swimbladder of this fish is cranially extended and makes connections with the inner ear (Dehadrai, 1957; Greenwood, 1963; Coombs and Popper, 1982). Furthermore, the lateral line canals on the head may be coupled to the inner ear via a flexible lateral window in the skull (Kapoor, 1964; Greenwood, 1973). This so called oto-physic connection is very similar to that found in Clupeomorpha (Wohlfahrt, 1936; Gray and Denton, 1979; Blaxter *et al.*, 1981), for which the lateral line has been found to be pressure sensitive (Blaxter *et al.*, 1981).

Origin of (low-pass) filtering characteristics of the hair cell's frequency selectivity function
The amplitude of the extracellular AC receptor potentials is partly shaped by the current through the voltage dependent K^+ channels (see Corey and Hudspeth, 1983a), which in turn are stimulated via the activity of the mechanosensitive transduction channels. Because of this membrane voltage dependence, the attenuation of the AC receptor potential can have its origin in the *RC* time constant of the hair cell membrane (Corey and Hudspeth, 1983a; Kroese *et al.*, 1980; Kroese and van Netten, 1989). The difference in (low-pass) cut-off frequency of the hair cell's frequency selectivity function between the ruffe and the knife fish then may be due to differences in *RC* time constants of the hair cell membranes in the two fish species. Such differences in filtering properties of hair cell membranes, maximising the AC response to relevant stimulus frequencies, have been reported for hair cells in different organs (cochlea, vestibule; reviewed by Correia, 1992), and for hair cells, tuned to different frequencies in the cochlea (Art and Fettiplace, 1987; Housley and Ashmore, 1992). In these organs, they arise from differences in total hair cell membrane area, resulting in differences in membrane capacitance, and from differences in (activated) membrane channel density, resulting in differences in membrane resistance (Art and Fettiplace, 1987; Correia, 1992; Housley and Ashmore, 1992).

The difference in temperature of 10 ts C at which the experiments of the ruffe (15 ^{o}C) and knife fish (25 ^{o}C) were performed may also influence the filtering properties of the hair cells directly. While the temperature dependence of the membrane capacitance is negligibly low (Cole, 1963), the conductances of membrane channels have Q_{10}'s of about 1.3 (Hille, 1992; Eatock and Manley, 1981). Thus, assuming that the *RC* time constant of the hair cell membrane determines the (low-pass) cut-off frequency ($f_{c,h}$), an increase of $f_{c,h}$ of about a factor 1.3 is expected for a 10 degrees increase in temperature. Since in the present experiments a factor of approximately 1.5 to 1.6 was found, the difference in cut-off frequency is probably not caused by the temperature difference only.

A larger dependence on temperature is expected if the gating kinetics of the transduction channels determines the (low-pass) cut-off frequency of the AC receptor potential. The rate constant of opening and closing of transduction channels is given by the Arrhenius equation (e.g. Corey and Hudspeth, 1983b; Gutfreund, 1995). It can be shown that, if channel gating causes the cut-off frequency of approximately 200 Hz at 15 ^{o}C, the Q_{10} of channel gating is expected to be approximately 2.2. Thus, at 25 ^{o}C the cut-off frequency would be approximately 440 Hz. Since this is higher than the cut-off frequencies of around 320 and 345 Hz found in our experiments, gating kinetics is not a likely candidate to determine the cut-off frequency of the hair cell transfer function.

It can thus be concluded that the difference in (low-pass) cut-off frequency between the frequency selectivity functions of the hair cells in the ruffe and the knife fish are probably caused by differences in *RC*-time constants of the hair cell membranes between the fish species. These differences in membrane time constants may be partly due to differences in environmental temperature. Differences in hair cell membrane area or in (activated) membrane channel density may also exist between the two fish species.

Conclusions

Cupular mechanics of head canal neuromasts in the lateral line of the African knife fish and clown knife fish is sensitive up to higher frequencies than in the ruffe. The frequency selectivity functions of the hair cells show that the filtering properties of the cell membranes are also higher and thus may be adapted to the mechanics. The extended bandwidth of both the mechanical frequency response of the cupula and the frequency selectivity of the hair cells suggest that the supraorbital canal neuromast in the knife fish is evolved to extract higher frequency components, up to 300 to 400 Hz, from natural stimuli.